

present invention provides an indication of bacterial strains reflecting the aberrant glycosylation (particularly in IBD patients) at the mucosal level. This can be monitored by a targeted qPCR test using stored faecal material. It is also rapid, simple (more practical as compared to biopsies) and low in cost (much cheaper than high throughput sequencing).

[0030] Specific embodiments and detail of aspects of the invention are now described with reference to the following figures wherein:

[0031] FIG. 1 shows a diagram of proposed pathways for the catabolism of sialic acid in *R. gnavus* ATCC 29149 and ATCC 35913. RgNanH releases 2,7-anhydro-Neu5Ac from α 2-3 linked sialylated substrates;

[0032] FIG. 2a is a diagram and graph of transcriptomic analysis of *R. gnavus* ATCC 29149 Nan cluster;

[0033] FIG. 2b shows *R. gnavus* ATCC 29149 nan operon analysis where 2b (a) is a diagram depicting the genomic organisation of the nan operon, and 2b (b) is a graph of qPCR analysis showing fold changes in expression of nan genes when *R. gnavus* was grown with 3'SL or 2,7-anhydro-Neu5Ac compared to glucose using $\Delta\Delta$ Ct calculation;

[0034] FIG. 3 shows the graphs of fluorescence emission spectrum of steady-state fluorescence analysis of ligand binding to RgSBP. 0.5 μ M RgSBP excited at 297 nm in the presence or absence of a) 2,7-anhydro-Neu5Ac or b) Neu5Ac. c) Titration of 0.5 μ M RgSBP with 2,7-anhydro-Neu5Ac. The data shown are representative of triplicate readings. d) Displacement of Neu5Ac with 2,7-anhydro-Neu5Ac;

[0035] FIG. 4 shows ITC isotherms of RgSBP binding to sialic acid derivatives where A) RgSBP binding to 2,7-anhydro-Neu5Ac and B) RgSBP binding to Neu5Ac;

[0036] FIG. 5 shows Sequence Similarity Networks (SSN) of predicted proteins in the *R. gnavus* nan cluster. Nodes representing proteins from *R. gnavus* strains (red) and *S. pneumoniae* strains (green) are highlighted. Clusters containing proteins from the nan cluster are shown using a dashed circle, a) InterPro family of sialidases, b) InterPro family of sialic acid aldolases, c) Top 2500 Blast hits of RgSBP, d) Top 2500 Blast hits of RUMGNA_02701, e) Top 2500 Blast hits of RUMGNA_02700, f) Top 2500 Blast hits of RUMGNA_02695;

[0037] FIG. 6 shows STD NMR analysis of the interaction of RgSBP with sialic acid, where a) STD NMR spectra of the interaction of

[0038] RgSBP (50 μ M) with a mixture of 2,7-anhydro-Neu5Ac (0.5 mM) and Neu5Ac (1 mM), with OFF-resonance reference spectra in red, difference spectra in blue. The resonances in the blue spectrum belong only to 2,7-anhydro-Neu5Ac demonstrating that RgSBP preferentially binds to 2,7-anhydro-Neu5Ac. b) Binding epitope mapping of 2,7-anhydro-Neu5Ac interacting with RgSBP. The initial slopes STD₀ (%) were normalized against the highest STD₀, assigned as 100%. The obtained factors were then classified as weak (0-60%), intermediate (60-80%), and strong (80-100%) and used to identify the close contacts found at the interface of binding c) Average DEEP STD factors for 2,7-anhydro-Neu5Ac obtained saturating RgSBP in spectral regions 0.6, 0.78, 1.44 ppm for aliphatic and 7.5, 7.23, 7.27 ppm for aromatic residues;

[0039] FIG. 7 *R. gnavus* sialic acid aldolase enzymatic reaction. a) Change of A_{340 nm} over time using *R. gnavus* sialic acid aldolase (RgNanA; black) or *E. coli* sialic acid aldolase (EcNanA; grey) with Neu5Ac (solid line) or 2,7-

anhydro-Neu5Ac (dashed line) reactions coupled to lactate dehydrogenase. b) Michaelis-Menten plot of RgNanA rate of reaction with increasing concentration of Neu5Ac. The rate of reaction at each concentration (μ M NADH) was determined in triplicate by measuring A_{340 nm} change using a standard curve. c) Cartoon representation of wild type RgNanA crystal structure showing the (β / α 8) TIM barrel organisation and Lys167 as yellow sticks. d) The RgNanA K167A active site is shown in orange with bound Neu5Ac in the open-chain ketone form shown in cyan. The green mesh represents the Neu5Ac Fo-Fc difference map at a sigma value of 3. Hydrogen bonding interactions are depicted using black dashed lines. In addition, the unbound RgNanA wt active site is shown in grey;

[0040] FIG. 8 shows RUMGNA_02695 catalyses the conversion of 2,7-anhydro-Neu5Ac to Neu5Ac, where a) HPLC analysis of DMB labelled RUMGNA_02695 reactions with 2,7-anhydro-Neu5Ac using different co-factors. NAD (black), NADH (pink), FAD (blue), no co-factor (brown), and a Neu5Ac standard (green). b) Michaelis-Menten plot of the rate of reaction for

[0041] RUMGNA_02695 with increasing concentration of 2,7-anhydro-Neu5Ac. The rate of reaction (μ M NADH) at each concentration was determined in triplicate by measuring A_{340 nm} change and using a standard curve;

[0042] FIG. 9 shows growth curves of a) *R. gnavus* ATCC 29149 (wild-type) and b) *R. gnavus* antisense mutant using the following sugars as sole carbon sources: media only (YCFA), 2,7-anhydro-Neu5Ac, 3'SL, Neu5Ac, glucose;

[0043] FIG. 10 shows the colonisation of germ-free C57BL/6J mice with *R. gnavus* ATCC 29149 wild-type or nan mutant strains. Mice were monocolonised with (a) *R. gnavus* wild-type (black) or nan mutant (red) strains individually or (b) in competition. Mice were orally gavaged with 1×10^8 of each strain, faecal samples were analysed at 3, 7 and 14 days after inoculation and caecal samples at 14 days after inoculation using qPCR. (c) Fluorescent in situ hybridisation (FISH) and immunostaining of the colon from *R. gnavus* monocolonised C57BL/6 mice. *R. gnavus* ATCC 29149 and *R. gnavus* nan mutant are shown in red. The mucus layer is shown in green and an outline of the mucus is shown in the first panels. Cell nuclei were counterstained with Sytox blue, shown in blue. Scale bar: 20 μ m. (d) Quantification of the distance between the leading front of bacteria and the base of the mucus layer. A total of 70 images of stained colon from 8 *R. gnavus* monocolonised mice were analysed;

[0044] FIG. 11 shows a schematic representation of gene organization in predicted homologs of the *R. gnavus* nan cluster. In the variety of in silico identified nan cluster homologs, the 37 *S. pneumoniae* cluster organisations are highly similar and represented here by the NanB cluster from *S. pneumoniae* D39. Cluster locus tag ranges are bracketed and genes are colour coded by predicted function as described in the inset; and

[0045] FIG. 12 shows a schematic of the indicated *R. gnavus* sialic acid metabolism pathway. RgNanH releases 2,7-anhydroNeu5Ac from α 2-3 linked sialylated glycoconjugates and is transported inside the bacterium via a 2,7-anhydro-Neu5Ac specific ABC transporter composed of a solute-binding protein (RgSBP) and two putative permeases. The 2,7-anhydro-Neu5Ac is then converted into Neu5Ac, by the action of an oxidoreductase (RgNanOx), before being catabolised into GlcNAc-6-P following the traditional path-